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Review Paper

Growth Factors and Antimicrobial Factors of Bovine Colostrum

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ABSTRACT

Colostrum is the first natural food produced by female mammals during the first 24–36h directly after giving birth. Chemically, colostrum is a very complex fluid rich in nutrients, antibodies and growth factors. In cows the antibodies provide passive immunity to the new born calf, whereas the growth factors especially stimulate the growth of the gut. The other antimicrobial components of colostrum include lactoferrin, lysozyme and lactoperoxidase. Bovine colostrum has also been used as a raw material for immunonoglubulin-rich commercial products (immune milk preparations). These products can be given orally to patients who are suffering infections of the gastrointestial tract or in order to prevent these infections. Usually, however, the cows have to be hyperimmunized against microorganisms, if specific antibodies are required. Several animal studies have shown that the growth factors in bovine colostrum, especially insulin-like growth factors, stimulate cell growth in the gut. Bovine colostrum is also known to contain insulin, transforming growth factor β and related growth factors, but their function in colostrum is not fully understood. Small amounts of these growth factors can also be detected in normal milk. Growth factors as well as antimicrobial factors of colostrum may be used as potential components in clinical nutrition in the future.

Keywords: colostrum; milk; growth factors; antimicrobial factors

INTRODUCTION

Colostrum is the first natural food for the newborn calf. It is secreted during the first few days after calving. The importance of colostrum for the health of calves has been known for a long time. Colostrum is not only a source of nutrients such as proteins, carbohydrates, fat, vitamins and minerals, but it also contains several biologically active molecules which are essential for specific functions. The most important bioactive components in colostrum include growth factors and antimicrobial factors. Growth factors promote the growth and development of the newborn calf while antimicrobial factors provide passive immunity and protect against infections during the first weeks of life. The antimicrobial activity of colostrum is due mostly to immunoglobulins, although colostrum also contains other antimicrobial factors such as lactoferrin, lysozyme and lactoperoxidase (reviews: Foley and Otterby, 1978; Reiter, 1978; Besser and Gay, 1994; Donovan and Odle, 1994; Shams, 1994). This review will focus on the above-mentioned bioactive components of bovine colostrum, including information on immune milk (from specifically hyperimmunized cows), an interesting pharmaceutical application based on colostral immunoglobulins.

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ANTIMICROBIAL COMPONENTS

Lactoferrin

Lactoferrin is an 80 kDa iron-binding glycoprotein present in colostrum, milk and to a lesser extent in other exocrine fluids such as tears. In addition to its antimicrobial activity, it has been proposed that lactoferrin plays a role in iron uptake in the intestine and the activation of phacocytes and immune responses. Receptors for lactoferrin are found on intestinal tissues, monocytes, macrophages, neutrophils, lymphocytes, platelets and on some bacteria (reviews: Reiter, 1978; Lönnerdahl and Iyer, 1995; Viljoen, 1995).

The cDNA for bovine lactoferrin has been isolated and the deduced amino acid sequence (708 amino acids) is homologous with human lactoferrin (68%) and human transferrin (60%), another iron-binding protein predominantly present in serum (Mead and Tweedie, 1990; Goodman and Schanbacher, 1991). The concentration of lactoferrin in bovine colostrum and mature milk is about 1.5–5 mg mL^{-1} and 0.1 mg mL^{-1}, respectively (Korhonen, 1977; Tsuji *et al.*, 1990).

Lactoferrin has been shown to inhibit the growth of several microbes, including *Escherichia coli* (Rainard, 1986; Saito *et al.*, 1991), *Salmonella typhimurium*, *Shigella dysenteria* (Batish *et al.*, 1988), *Listeria monocytogenes* (Payne *et al.*, 1990), *Streptococcus mutans* (Lassiter *et al.*, 1987), *Bacillus stearothermophilus* and *Bacillus subtilis* (Oram and Reiter, 1968). In a recent study it was shown that human and bovine lactoferrin and their N-terminal peptides were giardicidal against *Giardia lamblia in vitro* (Turchany *et al.*, 1995). It has been proposed that the antimicrobial effect of lactoferrin is based on its capacity to bind iron, which is essential for the growth of bacteria. However, recent studies have shown that in addition to iron chelation, other mechanisms are also involved. In fact, an antibacterial domain of bovine and human lactoferrin, distinct from the iron-binding region of the molecule, has been characterized (Saito *et al.*, 1991; Bellamy *et al.*, 1992).

Lactoferrin is active at neutral pH and in the presence of bicarbonate ions (Bullen *et al.*, 1972; Griffiths and Humphreys, 1977). Since bicarbonate is secreted into the lumen of the intestine, the conditions should be favourable for the antimicrobial activity of lactoferrin (Reiter, 1978). Lactoferrin has been shown to bind lipid A of lipopolysaccharides (LPS) (Appelmelts *et al.*, 1994) and cause the release of LPS from cell walls of bacteria (Ellison *et al.*, 1988; Yamauchi *et al.*, 1993a). In addition, lactoferrin binds to porin molecules in the outer membrane of *Escherichia coli* (Erdei *et al.*, 1994) and *Salmonella typhimurium* (Naidu and Arnold, 1994), resulting probably in permeability changes. Lactoferricin, a peptide derived from pepsin digestion of bovine lactoferrin, has antimicrobial activities against various bacteria and *Candida albicans* (Yamauchi *et al.*, 1993a; Jones *et al.*, 1994; Longhi *et al.*, 1994) and the interaction of lactoferrin with the cell surface is necessary for antimicrobial activity (Bellamy *et al.*, 1993). The results suggest that lactoferrin exerts its antimicrobial activity by modifying bacterial cell membranes. In addition to its antibacterial activity, lactoferrin has antiviral effects against herpes simples virus type-1 (HSV-1) (Fujihara and Hayashi, 1995), human immunodeficiency virus-1 (HIV-1) and human cytomegalovirus *in vitro* (Harmsen *et al.*, 1995).

Some data indicate that lactoferrin stimulates cell growth and acts as a growth factor or iron carrier molecule (Nichols *et al.*, 1987; Azuma *et al.*, 1989; Byatt *et al.*, 1990; Hagiwara *et al.*, 1995). In contrast, in other studies, lactoferrin could not substitute for transferrin (Amouric *et al.*, 1984) and it has been shown to inhibit cell growth (Rejman *et al.*, 1992; Hurley *et al.*, 1994). Recent studies show that lactoferrin can bind DNA and activate transcription, which might explain the molecular basis of growth regulation (Fleet, 1995). Thus, further studies are required to define the effects of lactoferrin on cell growth. The funtion of lactoferrin in iron absorption and immune responses has been discussed extensively in recent reviews (Lönnerdahl and Iyer, 1995; Viljoen, 1995).

Lysozyme

Lysozyme [EC.3.2.1.17] is a well-known antibacterial and lytic enzyme discovered by Fleming (1922). Lysozyme can be found in many mammalian body fluids, including colostrum. Especially rich sources of lysozyme include egg albumen and human milk. The natural substrate of the enzyme is the peptidoglycan layer of the bacterial cell wall and its degradation results in lysis of the bacteria (review: Reiter, 1978). Some recent results suggest that the antibacterial activity of lysozyme is due not only to its enzymatic activity, but also to its cationic and hydrophobic properties (Pellegrini *et al.*, 1992). The concentration of lysozyme in colostrum and in normal milk is about 0.14–0.7 and 0.07–0.6 $mg\,L^{-1}$, respectively (Korhonen, 1977). Milk lysozyme is active against a number of Gram-positive and some Gram-negative bacteria, which are completely resistant to egg white lysozyme (Vakil *et al.*, 1969). The presence of lactoferrin enhances the antibacterial activity of lysozyme against *E. coli* (Yamauchi *et al.*, 1993a), which also supports the hypothesis that lactoferrin damages the outer membrane of Gram-negative bacteria. Several genes encoding lysozymes (Steinhoff *et al.*, 1994; Irwin, 1995) have been found in a cow, and the purified lysozymes from cow kidney and stomach are different from cow's milk lysozyme (White *et al.*, 1988; Ito *et al.*, 1993). In addition, cow's milk lysozyme is strikingly different in amino acid content from human milk lysozyme and egg white lysozyme (Eitenmiller *et al.*, 1976). Further studies are required to define the exact functions of different types of lysozymes expressed even in a single animal.

Lactoperoxidase

Lactoperoxidase [EC 1.11.1.7] is a major antibacterial enzyme in colostrum. It is a basic glycoprotein containing a heme-group with Fe^{3+} and catalyzes the oxidation of thiocyanate (SCN^-) in the presence of hydrogen peroxide (H_2O_2), producing a toxic intermediary oxidation product. This product inhibits bacterial metabolism via the oxidation of essential sulphydryl groups in proteins (reviews: Reiter, 1978; Pruitt and Reiter, 1985).

The lactoperoxidase system protects the lactating mammary gland from infections caused by, for example, pathogenic *Streptococcus spp.* (Todhunter *et al.*, 1985; Marshall *et al.*, 1986). The lactoperoxidase system is also toxic to other Gram-positive and Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Salmonella typhimurium* (Reiter *et al.*, 1976), *Listeria monocytogenes* (Siragusa and Johnson, 1989; Kamau *et al.*, 1990; Gaya *et al.*, 1991), *Streptococcus mutans* (Thomas *et al.*, 1976,), *Staphylococcus aureus* (Kamau *et al.*, 1990) and psychrotrophic bacteria in milk (Björck, 1978). In addition, the lactoperoxidase system inactivates polio virus, vaccinia (Belding *et al.*, 1970) and human immunodeficiency virus type 1 (Yamaguchi *et al.*, 1993b) *in vitro*.

Bovine colostrum and milk contain about 11–45 $mg\,L^{-1}$ and 13–30 $mg\,L^{-1}$ lactoperoxidase, respectively (Korhonen, 1977). The gene encoding lactoperoxidase is expressed in epithelial cells of the lactating mammary gland, indicating that these cells secrete lactoperoxidase into milk (Cals *et al.*, 1994). The deduced amino acid sequence of the bovine lactoperoxidase gene is homologous to human myelo-, thyro- and eosinophil peroxidases. The single peptide

chain (612 amino acids) includes 15 half-cystines and 4–5 potential N-glycosylation sites, and the heme group is suggested to bind to the peptide chain via a disulphide linkage (Cals *et al.*, 1991). Bovine lactoperoxidase also contains a site with high affinity for calcium (Booth *et al.*, 1989). A lactoperoxidase-related enzyme devoid of the heme prosthetic group and enzyme activity has been purified from bovine milk, but its function is unknown (Dumontet and Rousset, 1983). The lactoperoxidase is partly activated by forming a complex with lysozyme and this interaction appears to be quite specific (Hulea *et al.*, 1989). The lactoperoxidase system and lactoferrin have been shown to have an additive, but not a synergistic, antibacterial effect against *Streptococcus mutans* (Soukka *et al.*, 1991).

Immunoglobulins

Maternal immunoglobulins are not transferred across the placenta to the fetus in cattle and calves are born with very low concentrations of serum immunoglobulins. Bovine colostrum is a very rich source of immunoglobulins and their absorption is essential to provide passive immunity after birth. These antibodies protect newborn calves against infectious enteric and respiratory diseases, which are principal reasons for mortality of calves. Calves with high serum immunoglobulin concentrations have lower mortality rates than calves with serum IgG $< 10\,g\,L^{-1}$ (Besser and Gay, 1994).

IgG_1 is the principal immunoglobulin type in colostrum whereas IgM, IgA and IgG_2 are present at considerably lower concentrations. The concentrations of immunoglobulins in colostrum are almost a hundred fold higher than in milk (Table 1).

Two processes are involved in the transfer of immunoglobulins from the cow to its calf. First, maternal immunoglobulins are absorbed from circulation and concentrated in the colostrum. Next, the colostral immunoglobulins are transferred from the lumen of the intestine into the circulation of the newborn calf. IgG is transported from blood into colostrum by an active receptor-mediated transfer across the mammary gland secretory epithelium in the dam. IgG diffuses across the vascular endothelium and binds to specific IgG-F_c receptors on the basal membrane of the mammary secretory epithelium. Pinocytotic vesicles transfer the immunoglobulin molecules through the epithelial cells, which secrete the molecules into colostrum. The transfer of IgG_1 to colostrum begins several weeks before and continues until the time of calving. This process results in a concentration of IgG_1 in colostrum that is 5- to 10-fold higher than in maternal serum (reviews: Bourne, 1977; Butler, 1983; Besser and Gay, 1994). In a newborn calf, the immunoglobulins are absorbed from colostrum into circulation via a non-selective macromolecular transport system across the small intestinal epithelium. No specific Ig receptors have been found to be associated with this process, which probably also transfer other macromolecules. This non-selective absorption occurs, however, only within about 24–36 h of birth, and provides the transmission of passive immunity from the cow to its calf (Bush and Stanley, 1980; Besser and Gay, 1994).

Table 1. The Concentration of Immunoglobulins in Colostrum and Normal Milk[a]

Immunoglobulin	Colostrum ($g\,L^{-1}$)	Normal milk ($g\,L^{-1}$)
IgG1	52.0–87.0	0.31–0.40
IgG2	1.6–2.1	0.03–0.08
IgM	3.7–6.1	0.03–0.06
IgA	3.2–6.2	0.04–0.06

[a]Mach and Pahud (1971).

Natural suckling usually provides sufficient immunoglobulins to calves. However, in cases where natural colostral intake is not possible or restricted, adequate amounts of immunoglobulins should be given. The most important factors influencing the passive transfer of immunoglobulins are the age of the calf and the mass of the antibodies consumed (Stott *et al.*, 1979; Stott and Fellah, 1983). The colostral immunoglobulin requirement of calves is estimated to be 80–100 g (Petrie, 1984) and the concentration of immunoglobulins should be at least $20\,g\,L^{-1}$ (Stott and Fellah, 1983). There are also commercial colostral supplements available for calves, but they usually contain low amounts of immunoglobulins compared to those in high-quality natural colostrum (Haines *et al.*, 1990).

IMMUNE MILK

As mentioned above, bovine colostrum is a rich source of natural immunoglobulins. The immunoglobulins from bovine colostrum at least partially retain biological activity in the human gastrointestinal tract (GI tract) (Roos *et al.*, 1995), and a lot of work has been done to prepare purified immunoglobulin fractions from colostrum for pharmaceutical use. If high amounts of specific antibodies are required, cows have to be hyperimmunized against specific microorganisms (Brüssow *et al.*, 1987). The hyperimmunization protocols usually include repeated subcutaneous, intramuscular and/or intravenous injections of vaccines. Immunoglobulin-rich fractions are usually prepared by removing fat and casein followed by concentration, sterilization and sometimes lyophilization or spray-drying. The resulting preparations (sometimes called immune milk preparations) contain high amounts of specific antibodies against the microorganisms in the vaccines. These preparations can then be given orally to patients suffering infections of the GI tract or in order to prevent these infections (Mietens *et al.*, 1979; Ebina *et al.*, 1983; Brüssow *et al.*, 1987; Tacket *et al.*, 1988). The following examples show that these immunoglobulin preparations from hyperimmunized cows have been used successfully in the treatment of these infections.

Orally administered anti-rotavirus immunoglobulins reduce the duration of rotavirus excretion and diarrhea (Hilpert *et al.*, 1987; Mitra *et al.*, 1995) and protect children against virus infection (Ebina *et al.*, 1983,; Davidson *et al.*, 1989). Tacket *et al.* (1992) showed that hyperimmune milk against *Shigella flexneri* prevents illness after a *Shigella* challenge, and Mietens *et al.*

(1979) and Tacket *et al.* (1988) showed that anti-*E. coli* immune milk is effective in eliminating enteropathogenic *E. coli* from the intestine. Hyperimmune bovine colostrum antibodies against *Cryptosporidium* have been shown to inhibit effectively the parasite infection *in vitro* (Flanigan *et al.*, 1991), in neonatal mice (Fayer *et al.*, 1990), in adult severe combined immune-deficient (SCID) mice (Riggs *et al.*, 1994) and in neonatal lambs (Naciri *et al.*, 1994). Immune milk has also been used successfully against *Cryptosporidium*-associated diarrhea in acquired immunodeficiency syndrome (AIDS) patients (Tzipori *et al.*, 1987; Nord *et al.*, 1990; Ungar *et al.*, 1990). Immune milk prepared against *Helicobacter pylori* has been shown to reduce the colonization of *Helicobacter pylori* in piglets (Cordle *et al.*, 1994), and there is some evidence that the anti-bacterial effect of anti-*Helicobacter pylori* immune milk may be mediated by complement (Korhonen *et al.*, 1994,). Deposition of complement components on *Streptococcus agalactiae* has also been shown to occur in normal bovine milk (Rainard and Poutrel, 1995). Colostral antibodies raised against *Clostridium difficile* toxins A and B protect hamsters against *Clostridium difficile* disease (Lyerly *et al.*, 1991).

The production costs and availability of immune milk products limit their use in food formulae, since in most cases immune milk must be administered daily. In addition, it has been shown that gastric acid and digestive enzymes reduce the neutralizing activity of bovine colostrum immunoglobulins (Petschow and Talbott, 1994). On the other hand, colostrum contains plasma-derived proteinase inhibitors, which might prevent degradation of bioactive components (Christensen *et al.*, 1995). It is usually not known whether they are present in immune milk preparations, but their addition to immune milk could enhance its efficacy. Thus, immune milk products may be valuable in special cases, e.g. in passive protection of hospitalized infants or AIDS patients against rotavirus and *Cryptosporidium* infection, when no other efficient treatment is available. If the production costs could be reduced, immune milk preparations may be used more widely as antimicrobial supplements in food formulae.

In addition to immunoglobulins, non-specific factors (lysozyme, lactoperoxidase, etc.) in hyperimmune milk also have positive synergistic antibacterial effects (Takashi *et al.*, 1992). Some interesting secondary effects of immune milk preparations have also been described. Skim milk from hyperimmunized cows has been demonstrated to have cholesterol and blood pressure lowering effects (Golay *et al.*, 1990; Sharpe *et al.*, 1994), but the mechanisms of these effects remain unknown and require further studies. Sharpe *et al.* (1994) suggested that increased amounts of IgG might result in changes in the human gut microflora, which might enhance the excretion of bile acids, leading to increased hepatic conversion of cholesterol into newly synthesized bile acids.

GROWTH FACTORS

Both normal milk and colostrum contain several peptide growth factors which stimulate the growth and differentiation of mammalian cells. The first indirect evidence of this was the finding that human milk (Klagsbrun, 1978) and bovine colostrum (Klagsbrun and Neumann, 1979) stimulated the growth of cultured mouse fibroblasts. Several reports confirmed these results and showed that bovine colostrum and its fractions stimulate the growth of many other types of mammalian cells *in vitro* (Klagsbrun and Neumann, 1979; Klagsbrun, 1980; Steimer *et al.*, 1981; Ramirez *et al.*, 1990; Iivanainen *et al.*, 1992; Pakkanen *et al.*, 1992,; Pakkanen and Neutra, 1994). Normal bovine milk shows much less stimulation, probably due to its lower content of growth factors (Klagsbrun and Neumann, 1979; Steimer *et al.*, 1981; Ramirez *et al.*, 1990). A summary of the published concentrations of the growth factors in colostrum and milk is shown in Table 2.

Table 2. The Concentration of Growth Factors in Colostrum and Normal Milk[a]

Growth factor	Colostrum ($\mu g\,L^{-1}$)	Milk ($\mu g\,L^{-1}$)
IGF-1	50–2000	< 10
IGF-2	200–600	< 10
TGF-β1	n.d.	4.3
TGF-β2	n.d.	n.d.
EGF	n.d.	< 2

[a]Results are collected from the text.

Insulin-like growth factors (IGF-1 and IGF-2)

The most abundant and best characterized growth factors in bovine colostrum are insulin-like growth factors (IGF-1 and IGF-2). IGFs are members of the insulin family of growth factors, consisting of insulin, IGF-1 (also known as somatomedin C), IGF-2 and relaxin. IGF-1 and IGF-2 are heat- and acid-stable and are widely distributed mediators of cellular growth, development and differentiation. IGFs are single-chain polypeptides of approximately 7.6 kDa. The polypeptide chain is composed of four polypeptide domains, denoted A, B, C and D, whereas insulin has no D domain and the C domain is cleaved post-translationally. Each IGF molecule contains three disulphide bridges (Baumrucker *et al.*, 1992; Baumrucker and Blum, 1993).

The biological effects of IGF-1 and IGF-2 are mediated primarily by a specific IGF receptor, type I IGF receptor, that is structurally homologous to insulin receptor. Another IGF receptor, type II IGF receptor (a cation-independent mannose-6-phosphate receptor) has a slightly higher affinity for IGF-2 than for IGF-1. Both receptors co-exist in many cells. IGFs, like insulin, stimulate glucose uptake, the synthesis of glycogen, protein, RNA, DNA and lipids and cell proliferation at nanomolar concentrations *in vitro*. *In vivo*, IGF-1 and IGF-2 are proposed to act both as endocrine hormones via the blood and locally as paracrine and autocrine growth factors. Six structurally related insulin-like growth factor binding proteins (IGFBs) are known in rats and humans, and they bind IGFs with high affinity and specificity. IGFBs are probably involved in the regulation of the biological activity of IGFs and two of them, IGFBP-2 and IGFBP-3, have been found in bovine milk (reviews:

Froesch *et al.*, 1985; Humbel, 1990; Baumrucker *et al.*, 1992; Baumrucker and Blum, 1993; Donovan and Odle, 1994).

IGF-I and IGF-2, together with a truncated form of IGF-1, –3N:IGF-1, have been purified to homogeneity from bovine colostrum. The amino acid sequence of the purified bovine IGF-1 is identical to that of human IGF-1 (Francis *et al.*, 1988; Marcotty *et al.*, 1991), and IGF-2 was found to differ in the three amino acid residues from human IGF-2. –3N:IGF-1 lacks the N-terminal tripeptide, Gly–Pro–Glu (Francis *et al.*, 1988). The IGF content of colostrum has been determined in several studies: ~200 $\mu g\,L^{-1}$ IGF-1 and ~200 $\mu g\,L^{-1}$ IGF-2 (Skaar *et al.*, 1991); 100–450 $\mu g\,L^{-1}$ IGF-1 (Oda *et al.*, 1989); 2000 $\mu g\,L^{-1}$ IGF-1 (Marcotty *et al.*, 1991); 50–150 $\mu g\,L^{-1}$ IGF-1 and 100–600 $\mu g\,L^{-1}$ IGF-2 (Malven *et al.*, 1987), 450–500 $\mu g\,L^{-1}$ IGF-1 (Vacher and Blum, 1993) whereas less than 10 $\mu g\,L^{-1}$ IGF-1 (Collier *et al.*, 1991) and IGF-2 (Vega *et al.*, 1991) has been found in normal bulk milk. Pasteurization of bovine milk (79°C, 45 s) does not alter the concentration of IGF-1, but the required treatment for infant formula, 121°C for 5 min, destroys the protein (Collier *et al.*, 1991). IGFs in colostrum and milk are supposed to originate from circulation and are not due to local synthesis in mammary tissues, although the exact mechanisms of their appearance in mammary secretions is unknown (Baumrucker and Blum, 1993).

Type I and II IGF receptors have been found in bovine mammary tissue (Dehoff *et al.*, 1988) and on intestinal epithelial cells (Laburthe *et al.*, 1988; Schober *et al.*, 1990; Baumrucker *et al.*, 1991), raising a question about the biological effects of IGFs in colostrum and milk. IGF-1 has been shown to be a potent stimulator of mitogenesis and galactopoesis of bovine mammary cells (Shamay *et al.*, 1988), suggesting that IGFs may also have effects on mammary tissue itself. On the other hand, dietary IGFs may have direct effects on epithelial cells of the GI tract or be absorbed into circulation and cause systemic effects. In the two latter cases, IGFs have to survive in the GI tract, where they are exposed to low pH and proteolytic enzymes. Dietary IGF-1 has indeed been demonstrated to stimulate cell proliferation in the GI tract of newborn piglets (Xu *et al.*, 1994) and calves (Baumrucker and Blum, 1993), and enhance D-xylose adsorption in newborn calves (Baumrucker *et al.*, 1994a). Dietary cow colostrum as such has also been shown to promote the growth of small intestine of newborn piglets (Tungthanathanich *et al.*, 1992). Baumrucker *et al.* (1992) showed that orally administered ^{125}I-IGF-1 was transported into the circulation, indicating that systemic effects are also possible. In fact, dietary IGF-1 has been shown to suppress erratic insulin secretion, stimulate prolactin secretion and cause a latent (4 days' delay after administration) increase in the concentration of IGF-1 in calf blood (Baumrucker and Blum, 1993). Xu and Wang (1996) showed that total and trichloroacetic acid (TCA)-precipitable radioactivity rose significantly in plasma in newborn and 3-day-old piglets 1 h after oral–gastric administration of ^{125}I-IGF-1. According to chromatographic analysis, ^{125}I-IGF-1 represented about 20% of the total plasma radioactivity in the newborn and 10% in the 3-day-old piglets. When the radioactivity of different tissues was analyzed, highest concentration of TCA-precipitable radioactivity was found in the stomach wall.

The truncated form of IGF-1, –3N:IGF-1, is especially potent *in vivo* and *in vitro*, possibly due to its reduced affinity for several IGFBPs (Ross *et al.*, 1989). In bioassays, –3N:IGF-1 has usually several-fold higher biological activity (depending on target cell type) than IGF-1 (Ballard, 1994), and subcutaneusly injected –3N:IGF-1 is about 2.5-fold more potent in increasing the body weight of rats than the full-length IGF-1 (Tomas *et al.*, 1992). Subcutaneously infused IGF-1 and especially –3N:IGF-1 induce substantial gut growth in rats, including all regions: stomach, small intestine and colon (Lemney *et al.*, 1991; Read *et al.*, 1992; Steeb *et al.*, 1994). The relative abundance (activity) of the three forms, IGF-1, IGF-2 and –3N:IGF-1, was estimated to be about 1:0.05:2, respectively, in bovine colostrum (Francis *et al.*, 1988), indicating that most of the IGF activity in colostrum is due to the presence of –3N:IGF-1.

It has been speculated that a bovine colostrum-based diet would provide natural growth stimulants such as IGFs in food. Some *in vivo* and *in vitro* studies support this idea. A dietary sterile-filtered colostrum-based food supplement has been demonstrated to increase serum IGF-1 in male athletes during a short-term strength and speed training period (Mero, 1995), and dietary colostrum has been shown to increase the concentration of blood IGF-1 in neonatal calves (Ronge and Blum, 1988; Grütter and Blum, 1991). Infused IGF-1 enhances muscle protein anabolism in human subjects (Fryburg *et al.*, 1995) and in rats (Tomas *et al.*, 1991a,). Purified IGF-1 acts as a survival factor in the protection of cultured Balb/c mouse fibroblasts against death (Tamm and Kikuchi, 1990) and stimulates amino acid uptake by cultured human placental trophoblasts (Karl, 1995). Colostrum as such also significantly inhibits protein degradation in different cultured mammalian cell lines (Ballard *et al.*, 1982).

What are the benefits of an IGF-rich diet such as colostrum for neonatal animals? Perhaps increased growth and turnover of the intestine provide a healthier gut, and increased uptake of dietary components and/or increased immunological performance and enhanced anabolism may stimulate growth generally. Unfortunately, there are no studies on the long-term effects of an IGF-1 positive/negative diet, which would clarify the role of IGF-1 in colostrum/milk.

Insulin

In addition to IGFs, varying amounts of insulin have been detected in normal bovine colostrum and milk: 10–50 $\mu g\,L^{-1}$ (Malven *et al.*, 1987); 85–327 $\mu g\,L^{-1}$ (Aranda *et al.*, 1991); and 20–25 $\mu g\,L^{-1}$ (Vacher and Blum, 1993). The highest concentration (327 $\mu g\,L^{-1}$) was found in the first milking and it fell to about 50% of its initial value 24 h post-partum. A stable concentration at about 14% of its initial value was reached 7 days post-partum (Aranda *et al.*, 1991). Insulin is taken up from the maternal circulation by the mammary gland, from which it is probably released

into colostrum (Malven *et al.*, 1987). Orally-administered insulin results in hypoglycemia in calves (Pierce *et al.*, 1964) and newborn pigs (Asplund *et al.*, 1962), indicating that insulin is absorbed and retains its biological activity in the GI tract. In addition, a colostrum diet increased the level of serum insulin in neonatal piglets (Burrin *et al.*, 1992) and calves (Shams and Eispanier, 1991). These results contradict two more recent studies where no increase in serum insulin or decline in blood glucose were detected after oral administration of insulin (Shulman, 1990; Grütter and Blum, 1991). Insulin receptors have been found in the membranes of intestinal epithelial cells (Fernandez-Moreno *et al.*, 1987; Gingerich *et al.*, 1987), indicating that insulin may also act directly on epithelial cells. This is supported by the finding that orally-administered insulin has been shown to increase ileal mucosal weight, protein, DNA and RNA content in newborn miniature pigs (Shulman, 1990).

Transforming growth factor beta (TGF-β1 and TGF-β2)

A very interesting growth factor found in bovine colostrum is transforming growth factor β (TGF-β). TGF-β is a highly pleiotropic growth factor with several different types of function. It stimulates proliferation of some cells, especially in connective tissue, whereas it acts as a growth inhibitor of some other cells, such as lymphocytes and epithelial cells. TGF-β plays an important role in embryogenesis, tissue repair, formation of bone and cartilage, and in the control of the immune system. Three isoforms of TGF-β (TGF-β1, TGF-β2 and TGF-β3) are known in humans and they are members of the TGF-β superfamily of peptide growth factors, including, for example, a family of bone morphogenic factors and a family containing activins and inhibins. Two high-affinity receptors and several other soluble and cell surface TGF-β-binding proteins are known to be involved in mediating the numerous effects of TGF-β (reviews: Rosen, 1989; Sporn and Roberts, 1992; Lin and Lodish, 1993).

The TGF-β2 gene, like other TGF-βs, encodes a large precursor molecule which is cleaved to an inactive precursor polypeptide chain. This polypeptide contains a large N-terminal domain and a C-terminal domain, which is the mature form of TGF-β2 (Madisen *et al.*, 1988). After proteolytic processing, the N-terminal domain remains non-covalently bound to the C-terminal domain and this complex is known as an inactive latent TGF-β2 (Miller *et al.*, 1992). Activation of the latent form by removal of the N-terminal domain is induced by changes in ionic strength, acidification or proteolytic enzymes (Meager, 1991). Most forms of TGF-β are homodimers containing two identical polypeptide chains, although heterodimers such as TGF-β1,2 and TGF-β2,3 have also been found (Ogawa *et al.*, 1992). The subunits of the active form of TGF-β are linked by a disulphide bond (Miyazono *et al.*, 1988).

TGF-β1 (Jin *et al.*, 1991) and TGF-β2 (Cox and Bürk, 1991; Jin *et al.*, 1991) have been purified from bovine milk. One of the TGF-β-related growth factors detected in colostrum (Tokuyama and Tokuyama, 1989; Tokuyama *et al.*, 1990) is probably indentical to TGF-β2 (Tokuyama and Tokuyama, 1993). Tokuyama and Tokuyama (1989) also found that colostrum contains a much higher level of TGF-β activity than normal milk. TGF-β2 is probably the predominant form in milk (Jin *et al.*, 1991; Cox and Bürk, 1991) and in colostrum (Tokuyama and Tokuyama, 1993). The whole amino acid sequence and 29 sequenced N-terminal amino acid residues of bovine TGF-β2 and TGF-β1, respectively (Jin *et al.*, 1991) were found to be identical with the corresponding sequences of human TGF-βs (Marquardt *et al.*, 1987), indicating remarkable conservation during evolution. Rogers *et al.* (1996) showed that the majority of TGF-β in bovine milk is present in a small protein complex with a molecular mass of 80 kDa. Using a bioassay (measuring the growth inhibition of mink lung epithelial cells) they detected 4.3 and 3.7 ng TGF-β mL^{-1} in bovine milk and cheese whey, respectively.

The function of TGF-βs in colostrum and milk is unknown. Cox and Bürk (1991) proposed that TGF-βs may be mediators of mucosal immunity and/or gut epithelial differentiation in the neonate. The first suggestion is supported by findings that TGF-β increases the production of IgG (Coffman *et al.*, 1989; Chen and Li, 1990; McIntyre *et al.*, 1993; Snapper *et al.*, 1993) and especially of IgA (Coffman *et al.*, 1989; Chen and Li, 1990) of lipopolysaccharide (LPS)-stimulated murine B lymphocytes. The stimulation of IgA production by TGF-β is due to isotype switching (Lebman *et al.*, 1990; Kunimoto *et al.*, 1992), and it is further enhanced by other lymphokines (IL-2 and IL-5) (Coffman *et al.*, 1989; Kim and Kagnoff, 1990; Lebman *et al.*, 1990). In addition, it has been demonstrated that TGF-β enhances expression of secretory component in rat epithelial cells, which is responsible for the transport of polymeric IgA into the intestinal lumen (McGee *et al.*, 1991). Since it is well known that IgA plays a major role in immunological protection of mucous membranes (Brown, 1978), it would be interesting to define the role of TGF-β in milk/colostrum in modulation of the immunological defence systems against pathogenic microorganisms in the gut. It should also be noted that intestinal epithelial cells produce TGF-β by themselves (Koyama and Podolsky, 1989). The suggestion that TGF-β is involved in the development of gut epithelium is supported by a finding that TGF-β1 induces terminal differentiation of intestinal epithelial cells *in vitro* (Kurokava *et al.*, 1987).

EPIDERMAL GROWTH FACTOR (EGF)

The EGF family of growth factors includes, for example, EGF, transforming growth factor-α (TGF-α) and amphiregulin. The most important members of this family are EGF and TGF-α, which can modulate the development of epidermis, mammary gland and gut and act as angiogenic factors. EGF and TGF-α bind to the same receptor, an MW 175 kDa cell surface glycoprotein with tyrosine kinase activity (reviews: Carpenter and Cohen, 1990; Tsuji *et al.*, 1990; Plaut, 1993; Donovan and Odle, 1994). There are contradictory results, however, about the presence of EGF in bovine colostrum. Brown and Blakeley (1983),

found a PDGF-like growth factor from mammary secretions of goats, sheep and cows that inhibited the binding of ^{125}I-labelled mouse epidermal growth factor (EGF) to mouse 3T3 fibroblasts, but stimulated cell proliferation. This activity was significantly higher in colostrum than in normal milk. Yagi *et al.* (1986) used human placental plasma membrane preparations as an EGF receptor source in a radioreceptor assay and estimated that raw cow's milk contained 324.2 ng mL^{-1} EGF receptor binding protein. On the other hand, Iacopetta *et al.* (1992) found that the use of human placental plasma membranes in the radioreceptor assay used gave erroneous results for bovine milk. They measured, using human epidermoid carcinoma cells (A431) as a receptor source, a very low concentration of EGF (less than 2 ng mL^{-1}) in bovine milk compared to human milk (30–40 ng mL^{-1}). In addition, Shing and Klagsbrun (1984) purified growth factors from both human and bovine milk, and found that the major growth factor of human milk, human milk growth factor III (HMGF III), was similar to EGF, but it was not present in bovine milk. Instead of EGF, the major growth factor they detected in bovine colostrum, colostrum-derived growth factor (BCGF), was shown to be structurally related to human platelet-derived growth factor (PDGF) (Shing and Klagsbrun, 1987; Shing *et al.*, 1987).

Other factors

In addition to growth factors, polypeptide growth inhibitors are also probably important in the regulation of cell proliferation. Brandt *et al.* (1988) purified a polypeptide factor from bovine milk that inhibits the growth of mammalian epithelial cells. Torre and Oliver (1988) showed that bovine colostrum inhibits blastogenesis of peripheral blood mononuclear cells.

Watson *et al.* (1992) found that bovine colostrum contains factor(s) that suppressed IgE antibody response. Ormrod and Miller (1991) detected an anti-inflammatory component in milk of hyperimmunized cows. Anti-inflammatory activity has also been found in human colostrum (Murphey and Buescher, 1993). Thus, it is obvious that bovine colostum contains immunomodulatory factors, which might, for example, prevent allergic responses to the multiplicity of novel food or other enviromental antigens.

DISCUSSION

Traditionally, bovine colostrum has been known to provide essential food for newborn calves. The role of immunoglobulins has been understood for a long time and it was later found that colostrum is also a rich source of growth factors and related molecules. Since it is relatively easy to collect large amounts of bovine colostrum, it has also been used as a raw material for industrial applications in recent years. For example, the commercial immune milk products available include Gastrogard (Northfield Laboratories, Oakden, Australia), a product used to prevent diarrhea caused by rotavirus in young children and PR$_x$O-IMMUNE™ 99 (GalaGen Inc., Minnesota, USA), a product used on young calves to prevent scours caused by *E. coli*. There are also several milk- or colostrum-derived oral supplements for newborn calves in which colostral intake is uncertain or known to be inadequate. However, some of these products contain very low immunoglobulin concentrations compared to those found in normal high-quality raw colostrum (Haines *et al.*, 1990). Biotest Pharma GmbH (Frankfurt, Germany) produces Lactimmunglobulin Biotest, a product for human subjects which contains immunoglobulins from colostrum of non-immunized cows. It has been tested, for example, in the treatment of severe diarrhea in AIDS patients (Stephan *et al.*, 1990). Some attempts have been made to incorporate the lactoperoxidase system into toothpaste (Biotene), but no bactericidal effect against salivary *Streptococcus spp.* or *Lactobacillus spp.* was observed (Lenander-Lumikari *et al.*, 1993; Kirstilä *et al.*, 1994). Viable Bioproducts Ltd (Turku, Finland) produces Bioenervi™, a sterile-filtered colostrum-based product, which is designed to provide growth factors and antimicrobial factors during strenuous physical activity, e.g. training of athletes (Mero, 1995). More bovine colostrum-based products will probably become available in the future, since a lot of research on this subject is ongoing. Thus, both basic and applied research into bovine colostrum will expand in the future.

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